



FIGURE 1a

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)

*Escherichia coli* B Phytase Sequence

1-  
ATG AAA GCG ATC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG  
Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro  
  
CAA TCT GCA TTC GCT CAG AGT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG  
Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Glu Ser Val Val  
  
ATT GTC AGT CGT CAT GGT GTG CGT GCT CCA ACC AAG GCC ACG CAA CTG ATG  
Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met  
  
CAG GAT GTC ACC CCA GAC GCA TGG CCA ACC TGG CCG GTA AAA CTG GGT TGG  
Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp  
  
CTG ACA CCG CGN GGT GGT GAG CTA ATC GCC TAT CTC GGA CAT TAC CAA CGC  
Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg  
  
CAG CGT CTG GTA GCC GAC GGA TTG CTG GCG AAA AAG GGC TGC CCG CAG TCT  
Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser  
  
GGT CAG GTC GCG ATT ATT GCT GAT GTC GAC GAG CGT ACC CGT AAA ACA GGC  
Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly  
  
GAA GCC TTC GCC GCC GGG CTG GCA CCT GAC TGT GCA ATA ACC GTA CAT ACC  
Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr  
  
CAG GCA GAT ACG TCC AGT CCC GAT CCG TTA TTT AAT CCT CTA AAA ACT GGC  
Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn Pro Leu Lys Thr Gly  
  
GTT TGC CAA CTG GAT AAC GCG AAC GTG ACT GAC GCG ATC CTC AGC AGG GCA  
Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala  
  
GGA GGG TCA ATT GCT GAC TTT ACC GGG CAT CGG CAA ACG GCG TTT CGC GAA  
Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu  
  
CTG GAA CGG GTG CTT AAT TTT CCG CAA TCA AAC TTG TGC CTT AAA CGT GAG  
Leu Glu Arg Val Leu Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu  
  
AAA CAG GAC GAA AGC TGT TCA TTA ACG CAG GCA TTA CCA TCG GAA CTC AAG  
Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys  
  
GTG AGC GCC GAC AAT GTC TCA TTA ACC GGT GCG GTA AGC CTC GCA TCA ATG  
Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met  
  
CTG ACG GAG ATA TTT CTC CTG CAA CAA GCA CAG GGA ATG CCG GAG CCG GGG  
Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly  
  
TGG GGA AGG ATC ACC GAT TCA CAC CAG TGG AAC ACC TTG CTA AGT TTG CAT  
Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His  
  
AAC GCG CAA TTT TAT TTG CTA CAA CGC ACG CCA GAG GTT GCC CGC AGC CGC  
Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg  
  
GCC ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCC CAT CCA CCG  
Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro



RECOMBINANT BACTERIAL PHYTASES AND USES  
THEREOF

FIGURE 1b

CAA AAA CAG GCG TAT GGT GTG ACA TTA CCC ACT TCA GTA CTG TTT ATT GCC  
Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu Phe Ile Ala

GGA CAC GAT ACT AAT CTG GCA AAT CTC GGC GGC GCA CTG GAG CTC AAC TGG  
Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu Glu Leu Asn Trp

ACG CTT CCC GGT CAG CCG GAT AAC ACG CCG CCA GGT GGT GAA CTG GTG TTT  
Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly Gly Glu Leu Val Phe

GAA CGC TGG CGT CGG CTA AGC GAT AAC AGC CAG TGG ATT CAG GTT TCG CTG  
Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln Trp Ile Gln Val Ser Leu

GTC TTC CAG ACT TTA CAG CAG ATG CGT GAT AAA ACG CCG CTG TCA TTA AAT  
Val Phe Gln Thr Leu Gln Gln Met Arg Asp Lys Thr Pro Leu Ser Leu Asn

ACG CCG CCC GGA GAG GTG AAA CTG ACC CTG GCA GGA TGT GAA GAG CGA AAT  
Thr Pro Pro Gly Glu Val Lys Leu Thr Leu Ala Gly Cys Glu Glu Arg Asn

GCG CAG GGC ATG TGT TCG TTG GCA GGT TTT ACG CAA ATC GTG AAT GAA GCA  
Ala Gln Gly Met Cys Ser Leu Ala Gly Phe Thr Gln Ile Val Asn Glu Ala

CGC ATA CCG GCG TGC AGT TTG AGA TCT CAT CAC CAT CAC CAT CAC TAA 1323  
Arg Ile Pro Ala Cys Ser Leu Arg Ser His His His His His His End



**FIGURE 2**  
**pH/Temperature Profile and Stability**

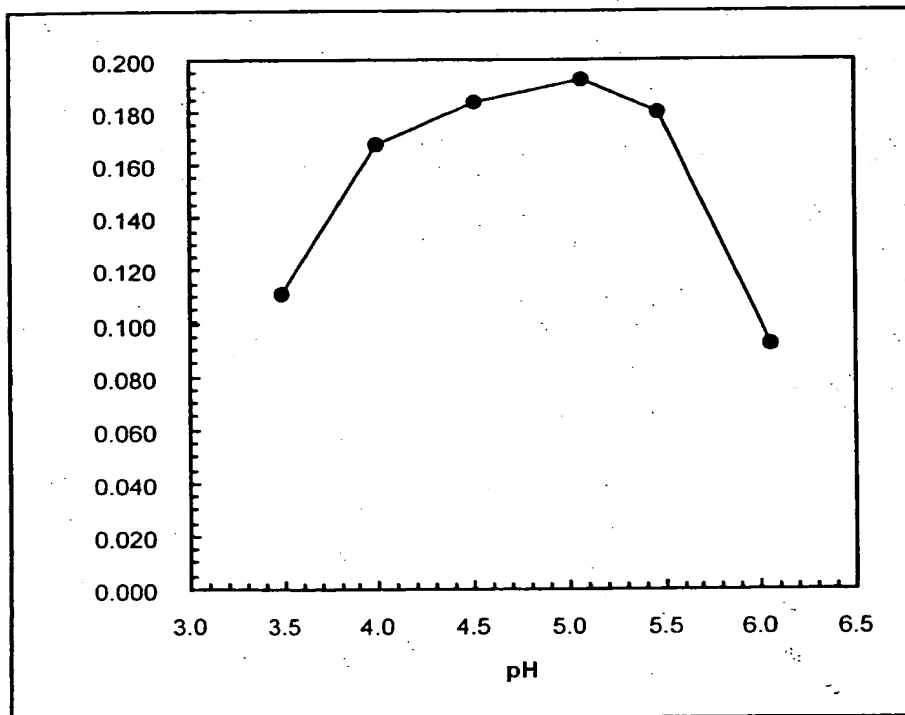
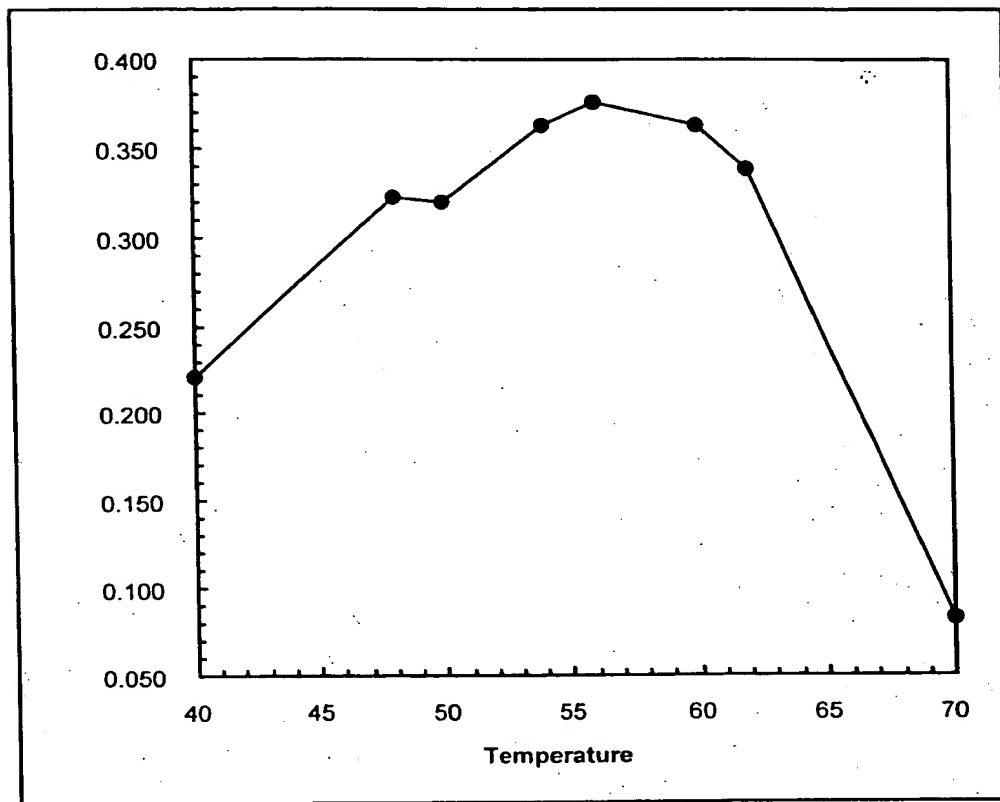




Figure 3:

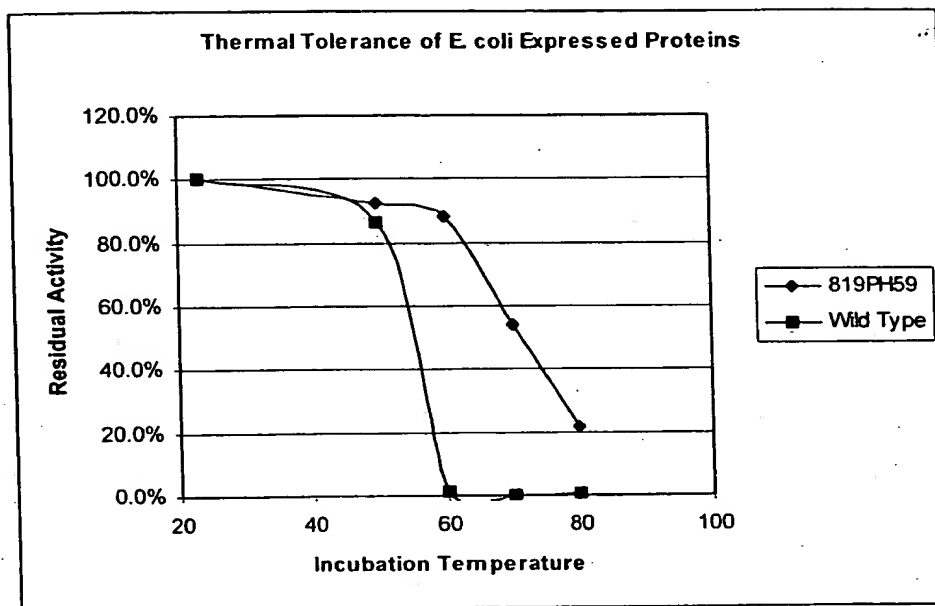




Figure 4

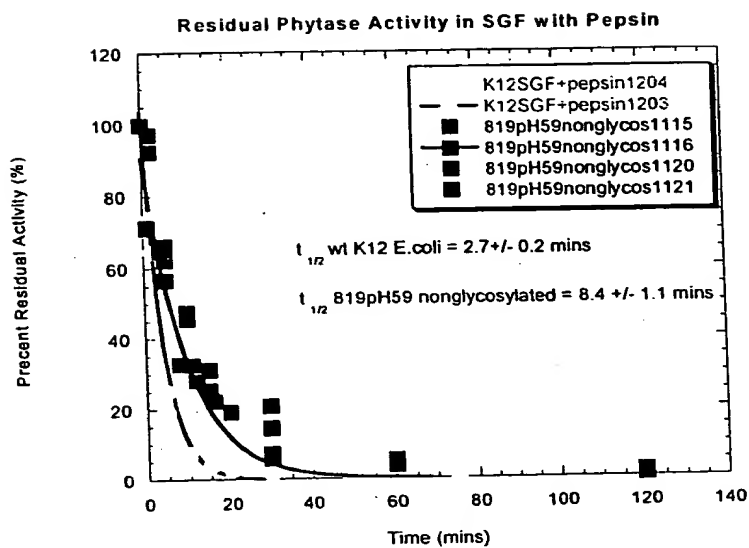




Figure 5:

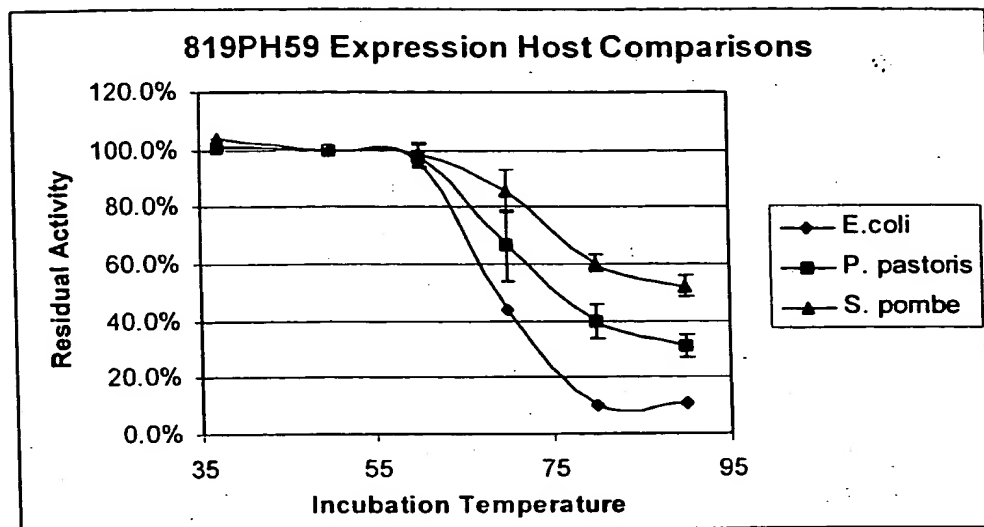




Figure 6.

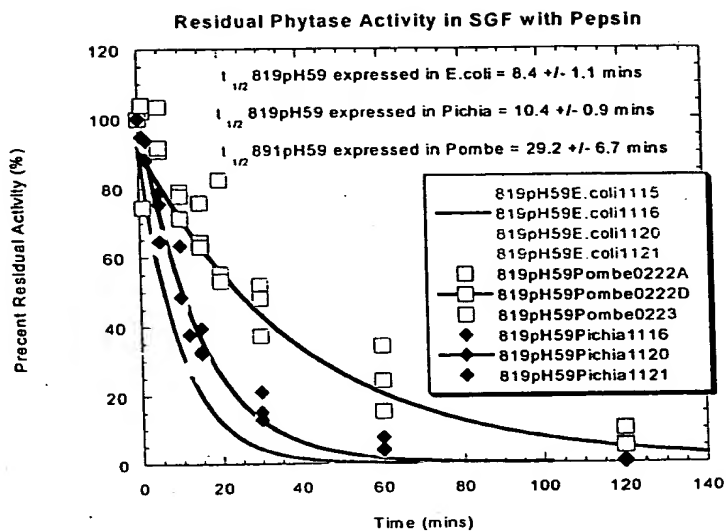




Figure 7a

E. coli appA (GenBank accession no. M58708 ) (SEQ ID NO:7)

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1 taaggagcag aaacaatgtg gtatttactt tggctcgtcg gcattttgtt gatgtgttcg
61 ctctccaccc ttgtgttggt atggctggac ccgcgtctga aaagttaacg aacgtaggcc
121 tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
181 catatcgatg aaagcatct taatccatt ttatctctt ctgattccgt taaccccgca
241 atctgcattc gctcagagt agccggagct gaagctggaa agtgtggtga ttgtcagtcg
301 tcatggtgtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca cccagacgc
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cgnggtggtg agctaactcg
421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaagg
481 ctgcccgcag tctggtcagg tcgcgattat tctgatgtc gacgagcgt cccgtaaaac
541 aggcgaagcc ttcgccgccg ggctggcacc tgactgtgca ataaccgtac ataccaggc
601 agatacgtcc agtcccgtc cgttatttaa tcctctaaaa actggcgtt gccaaactgga
661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtaattg ctgactttac
721 cgggcatcgg caaacggcgt ttcgcgaact ggaacgggtg ctaatttc cgcaatcaaa
781 ctgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
841 ggaactcaag gtgagcgccg acaatgtctc attaacgggt gcggtagcc tcgcatcaat
901 gctgacggag atattctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
961 gatcaccgat tcacaccagt ggaacacctt gctaagttg cataacgcgc aatttiatt
1021 gctacaacgc acgccagagg ttgccgcag ccgcgccacc ccgttattag atttgatcaa
1081 gacagcgttg acgccccatc caccgcaaaa acaggcgtat ggtgtgacat taccacttc
1141 agtgcgtgtt atgcgccgac acgatactaa tctggcaaat ctgcggcgcg cactggagct
1201 caactggacg ctcccggtc agccggataa cacgccgcca ggtggtgaac tgggtttga
1261 acgctggcgt cggctaagcg ataacagcca gtggattcag gttcgttg tcttcagac
1321 ttacagcag atgcgtgata aaacgccgct gtcattaaat acgccgccg gagaggtgaa
1381 actgaccctg gcaggatgtg aagagcgaag tgcgcagggc atgtgttcgt tggcaggttt
1441 tacgcaaac gtgaatgaag cacgcatacc ggcgtgcagt ttgtaatgca taaaaagag
1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
1561 ccggaaaagg cgttcacgcc gcacccggcc acttcagti ttctcttc tcggagtaac
1621 tataaccgta atagttag ccgtaactgt aagcgggtgt ggccggtta atcacaccat
1681 tgaggatagc gccttaata ttgacgcctg cctgttcag acgtgcatt gacaaactca
1741 cctcttggc ggtgtcaag ccaaacgcg caaccagcag gctggtgcca acagaacgcc
1801 ccacgaccgc ggcatcactc accgccagca tcggcggcgt atcgacaatc accagatcgt
1861 aatggtcgtt cgccatttc agtaatgac gcacccgac g
```





Figure 7b

1 taaggagcag aaacaatgtg gtatttactt tgggtcgtcg gcattttgtt gatgtgttcg  
61 ctctccacc ttgtttggt atggctggac ccgctctga aaagtaacg aacgtaggcc  
121 tgatcgggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcgga  
181 catatcgatg aaagcgatct taatccatt ttatctctt ctgattccgt taaccccgca  
241 atctgcattc gctcagagt agccggagct gaagctggaa agtgtgtga ttgtcagtcg  
301 tcatggtgtg cgtgctcaa ccaaggccac gcaactgatg caggatgtca cccagacgc  
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cnggtgttg agctaactgc  
421 ctatctcgga cattaccaac gccagcgtct gtagccgac ggattgctgg cgaaaaagg  
481 ctgcccgcag tctggtcagg tcgcgattat tgcgtatgtc gacgagcgt cccgtaaaa  
541 aggcgaagcc ttcgcccg ggctggcacc tgactgtgca ataaccgtac ataccaggc  
601 agatacgtcc agtccgcatc cgttatttaa tctctaaaa actggcggtt gccactgga  
661 taacgcgaac gtgactgacg cgtactcag caggcgagga ggtcaattg ctgacttac  
721 cgggcatcgg caaacggcgt ttcgcgaact ggaacgggtg cttaatttc cgcaatcaa  
781 ctgtgcctt aaacgtgaga aacaggacga aagctgtca ttaacgcagg cattaccatc  
841 ggaactcaag gtgagcgccg acaatgtctc attaacgggt gcgtaagcc tcgcatcaat  
901 gctgacggag atatttcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag  
961 gatcacgat tcacaccagt ggaacacctt gctaagtgtg cataacgcgc aattttatt  
1021 gctacaacgc acgccagagg ttgccgcag ccgcgccacc ccgtattag attgatcaa  
1081 gacagcgtt acgccccatc caccgcaaaa acaggcgtat ggtgtgacat taccacttc  
1141 agtctgttt atcgccggac acgatactaa tctggcaaat ctgcggcgcg cactggagct  
1201 caactggacg ctccccgtc agccggataa cacgccgcca ggtggtgaac ttgtgttga  
1261 acgtggcgt cggctaagcg ataacagcca gtggattcag gttcgttg tcttcagac  
1321 ttacagcag atgcgtgata aaacgccgt gtcattaaat acgccgccg gagaggtaga  
1381 actgacctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgtcgt tggcaggtt  
1441 tacgcaaatc gtgaatgaag cagcataacc ggcgtgcagt ttgtaatgca taaaaagag  
1501 cattcagtta cctgaatgct ctgaggtga tgacaaacga agaactgtct aatgcgtaga  
1561 ccggaagg cgttcacgcc gcatccgcc acttcagtt ttcctttc tcggagtaac  
1621 tataaccgta atagttag ccgtaactgt aagcgggtct ggcggttata atcacaccat  
1681 tgaggatagc gccttaata ttgacgcctg cctgtccag acgtgcatt gacaaactca  
1741 cctcttggc ggtgtcaag ccaaacgcg caaccagcag gctggtgcca acagaacgcc  
1801 ccacgaccgc ggcatcactc accgccagca tcggcgcggt atcgacaatc accagatcgt  
1861 aatggtcgtt cgccattcc agtaattgac gcatccgatc g



Figure 8

Amino acid sequence for E. coli appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT  
PDAWPTWVPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQVA  
IADVDERTTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA  
NVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ  
ALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNT  
LLSLHNAQFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLF  
AGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWQVSL  
VFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARI  
PACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme  
(SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT  
PDAWPTWVPVKLGELTPRGGELIAYLGHYWRQRLVADGLLPKCGCPQSGQVAI  
IADVDERTTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA  
NVTDAILERAGGSIADFTGHYQTAFRELERVLNFPQSNLCLKREKQDESCSLTQ  
ALPSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNT  
LLSLHNAQFDLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLF  
AGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWQVSL  
VFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARI  
PACSL